

IN THE CLAIMS:

For purposes of this Supplemental Response, Applicants present the pending claims incorporating the claim amendments made in applicants' last Response and Amendment:

- 1.-26. Cancelled.
27. (Previously presented) A method for detecting cytosine methylation and methylated CpG islands within a genomic sample of DNA comprising:
 - (a) contacting a genomic sample of DNA with a modifying agent that modifies unmethylated cytosine to produce a converted nucleic acid;
 - (b) amplifying the converted nucleic acid by means of oligonucleotide primers in the presence of one or a plurality of specific oligonucleotide probes, wherein one or a plurality of the oligonucleotide primers or the specific probe(s) are capable of distinguishing between unmethylated and methylated nucleic acid, with the proviso that at least one oligonucleotide probe is a CpG-specific probe capable of distinguishing between unmethylated and methylated nucleic acid; and
 - (c) detecting, in real-time during the amplification, the methylated nucleic acid based on amplification-mediated probe displacement.
28. (Original) The method of claim 27 wherein the amplifying step is a polymerase chain reaction (PCR).
29. (Original) The method of claim 27 wherein the modifying agent is bisulfite.
30. (Original) The method of claim 27 wherein the converted nucleic acid contains uracil in place of unmethylated cytosine residues present in the unmodified nucleic acid-containing sample.
31. (Original) The method of claim 27 wherein the probe further comprises one or a plurality of fluorescence label moieties.
32. (Original) The method of claim 31 wherein the amplification and detection step comprises fluorescence-based quantitative PCR.
33. (Previously presented) The method of claim 31, wherein the probe is a FRET probe, or a dual-label probe comprising a fluorescence-reporter moiety and fluorescence-quencher moiety.
34. (Previously presented) The method of claim 33, wherein the FRET probe is one component of a real-time PCR hybridization probe pair.
35. (Previously presented) The method of claim 33, wherein the probe is a nuclease cleavable dual-label probe.
36. (Original) The method of claim 27, wherein at least one of the primers comprises a CpG-specific probe.

37. (Cancelled).
38. (Previously presented) A method for detecting a methylated CpG-containing nucleic acid comprising:
- (a) contacting a nucleic acid-containing sample with a modifying agent that modifies unmethylated cytosine to produce a converted nucleic acid;
 - (b) amplifying the converted nucleic acid in the sample by means of oligonucleotide primers in the presence of a CpG-specific oligonucleotide probe, wherein the CpG-specific probe, but not the primers, distinguishes between modified unmethylated and methylated nucleic acid; and
 - (c) detecting, in real-time during the amplification, the methylated nucleic acid based on amplification-mediated probe displacement.
39. (Original) The method of claim 38 wherein the amplifying step comprises a polymerase chain reaction (PCR).
40. (Original) The method of claim 38 wherein the modifying agent comprises bisulfite.
41. (Original) The method of claim 38 wherein the converted nucleic acid contains uracil in place of unmethylated cytosine residues present in the unmodified nucleic acid-containing sample.
42. (Original) The method of claim 38 wherein the probe further comprises one or a plurality of fluorescence label moieties.
43. (Original) The method of claim 42 wherein the amplification and detection step comprises fluorescence-based quantitative PCR.
44. (Previously presented) The method of claim 42, wherein the probe is a FRET probe, or a dual-label probe comprising a fluorescence-reporter moiety and fluorescence-quencher moiety.
45. (Previously presented) The method of claim 44, wherein the FRET probe is one component of a real-time PCR hybridization probe pair.
46. (Previously presented) The method of claim 44, wherein the probe is a nuclease cleavable dual-label probe.
47. (Original) The method of claim 38, wherein at least one of the primers comprises a CpG-specific probe.
48. (Cancelled).
49. (Original) The method of claim 38 wherein methylation amounts in the nucleic acid sample are quantitatively determined based on reference to a control reaction for amount of input nucleic acid.
50. (Previously presented) A method for detecting a methylated CpG-containing nucleic acid comprising:

- (a) contacting a nucleic acid-containing sample with a modifying agent that modifies unmethylated cytosine to produce a converted nucleic acid;
- (b) amplifying the converted nucleic acid in the sample by means of oligonucleotide primers in the presence of a CpG-specific oligonucleotide probe, wherein both the primers and the CpG-specific probe distinguish between modified unmethylated and methylated nucleic acid; and
- (c) detecting, in real-time during the amplification, the methylated nucleic acid based on amplification-mediated probe displacement.

51. (Original) The method of claim 50 wherein the amplifying step comprises a polymerase chain reaction (PCR).

52. (Original) The method of claim 50 wherein the modifying agent is bisulfite.

53. (Original) The method of claim 50 wherein the converted nucleic acid contains uracil in place of unmethylated cytosine residues present in the unmodified nucleic acid-containing sample.

54. (Original) The method of claim 50 wherein the probe further comprises one or a plurality of fluorescence label moieties.

55. (Original) The method of claim 54 wherein the amplification and detection step comprises fluorescence-based quantitative PCR.

56. (Previously presented) The method of claim 54, wherein the probe is a FRET probe, or a dual-label probe comprising a fluorescence-reporter moiety and fluorescence-quencher moiety.

57. (Previously presented) The method of claim 56, wherein the FRET probe is one component of a real-time PCR hybridization probe pair.

58. (Previously presented) The method of claim 56, wherein the probe is a nuclease cleavable dual-label probe.

59. (Original) The method of claim 50, wherein at least one of the primers comprises a CpG-specific probe.

60. (Cancelled).

61. (Previously presented) A method for detecting cytosine methylation within a genomic sample of DNA, comprising: obtaining a methylation kit; conducting, using the kit, a real-time methylation assay of the genomic DNA sample; and determining, based on the methylation assay, whether cytosine methylation is present in the DNA sample, and wherein the methylation kit comprises:

a modifying agent that modifies unmethylated cytosine to produce a converted nucleic acid;

primers for amplification of the converted nucleic acid;

primers for the amplification of control unmodified nucleic acid; and

a CpG-specific probe the detection of which, during the amplification of the converted nucleic acid, is in real-time based on amplification-mediated probe displacement, wherein the CpG-specific probe distinguishes between modified unmethylated and methylated nucleic acid, and wherein the primers each may or may not distinguish between unmethylated and methylated nucleic acid.

62. (Previously presented) The method of claim 61, wherein the modifying agent is bisulfite.

63. (Previously presented) The method of claim 61 wherein the modifying agent converts cytosine residues to uracil residues.

64. (Previously presented) The method of claim 61, wherein the CpG-specific probe, but not the primers for amplification of the converted nucleic acid, distinguishes between modified unmethylated and methylated nucleic acid.

65. (Previously presented) The method of claim 61, wherein both the CpG-specific probe, and the primers for amplification of the converted nucleic acid, distinguish between modified unmethylated and methylated nucleic acid.

66. (Previously presented) The method of claim 61, wherein the CpG-specific probe further comprises one or a plurality of fluorescence label moieties.

67. (Previously presented) The method of claim 66, wherein the CpG-specific probe is a FRET probe, a real-time PCR hybridization probe, or a dual-label probe.

68. (Previously presented) The method of claim 61, wherein one of the primers for amplification of the converted nucleic acid comprises the CpG-specific probe.

69. (Cancelled).

70. (Previously presented) The method of claim 27, wherein detecting based on amplification-mediated probe displacement, comprises detecting amplification-mediated change of probe fluorescence.

71. (Previously presented) The method of claim 38, wherein detecting based on amplification-mediated probe displacement, comprises detecting amplification-mediated change of probe fluorescence.

72. (Previously presented) The method of claim 50, wherein detecting based on amplification-mediated probe displacement, comprises detecting amplification-mediated change of probe fluorescence.

73. (Previously presented) The method of claim 61, wherein detecting based on amplification-mediated probe displacement, comprises detecting amplification-mediated change of probe fluorescence.